Histone Deacetylase 6 Associates With Ribosomes and Regulates De Novo Protein Translation During Arsenite Stress

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Histone deacetylase 6 (HDAC6) is known as a cytoplasmic enzyme that regulates cell migration, cell adhesion, and degradation of misfolded proteins by deacetylating substrates such as α-tubulin and Hsp90. When HaCaT keratinocytes were exposed to 1–200 μM sodium arsenite, we observed perinuclear localization of HDAC6 within 30 min. Although the overall level of HDAC6 protein did not change, sodium arsenite caused an increase of HDAC6 in ribosomal fractions. Separation of ribosomal subunits versus intact ribosomes or polysomes indicated that HDAC6 was mainly detected in 40/43S fractions containing the small ribosomal subunit in untreated cells but was associated with 40/43S and 60/80S ribosomal fractions in arsenite-treated cells. Immunocytochemistry studies revealed that arsenite caused colocalization of HDAC6 with the ribosomal large and small subunit protein L36a and S6. Both L36a and S6 were detected in the immunocomplex of HDAC6 isolated from arsenite-treated cells. The observed physical interaction of HDAC6 with ribosomes pointed to a role of HDAC6 in stress-induced protein translation. Among arsenite stress–induced proteins, de novo Nrf2 protein translation was inhibited by Tubastatin A. These data demonstrate that HDAC6 was recruited to ribosomes, physically interacted with ribosomal proteins, and regulated de novo protein translation in keratinocytes responding to arsenite stress.

Key Words: Nrf2; protein translation; ribosomes; subcellular relocalization.

Low to mild doses of ROS induce cell survival response, including transcription of a cluster of antioxidant and detoxification genes. A master switch for these antioxidant and detoxification genes is the nuclear factor erythroid-2–related factor (Nrf2), a member of the cap’n’collar family of bZIP transcription factors (Ishii et al., 2002; Pi et al., 2003). Cytoplasmic Nrf2 is bound with kelch-like ECH-associated protein 1 (KEAP 1), which prevents nuclear translocation of Nrf2 and mediates ubiquitination thus degradation of Nrf2 protein. Upon oxidative stress, Nrf2 dissociates from KEAP 1 and translocates to the nucleus, where it activates transcription of the genes containing the antioxidant response element in the promoter (Ishii et al., 2002). Examples of such genes include glutathione S-transferase, NAD(P)H quinone oxidoreductase 1, microsomal epoxide hydrolase, γ-glutamylcysteine synthetase, ferritin, heme oxygenase-1, and peroxiredoxins (Ishii et al., 2002, 2004). Whereas Nrf2 dissociation from KEAP 1 is a well-established event for Nrf2 protein stabilization, recent works from our laboratory have demonstrated that oxidative stress causes de novo translation of Nrf2 protein, providing a novel mechanism of cytoprotection involving Nrf2 (Purdom-Dickinson et al., 2007b; Xu, Zhang, Sheveleva, Strom, Dinh, Lee, and Chen, unpublished data; Zhang et al., 2011). However, it is commonly known that physical or chemical stresses cause an inhibition of protein synthesis in general. How Nrf2 gene escapes the general control of protein synthesis and undergoes de novo protein translation under stress conditions remains unclear.

Posttranslational modifications play an important role in regulating protein functions. Reversible acetylation of histones provides a mean to regulate chromatin structure and consequently gene transcription. Although many acetylases have been found to catalyze lysine acetylation of histones and are therefore named histone acetylases (HATs), some of these HATs can also acetylate nonhistone proteins. The process of protein deacetylation is carried by deacetylases collectively named histone deacetylases (HDACs). HDACs are divided into three classes: Class I, including HDAC1, 2, 3, and 8, is homologous to the yeast protein reduced potassium dependency...
formation in human HaCaT keratinocytes, we found that stress granules, an aggregate of stalled protein translation, operate to preserve mRNA healing. One report indicates that HDAC6 is a component of the cytoplasmic retention of HDAC6 (Yang and Gregoire, 2005). Class III HDACs, also termed the signal information regulators (SIRT1-7), differ from other classes of deacetylases by nicotinamide adenine dinucleotide dependent activity (Yang and Gregoire, 2005).

HDAC6 was first reported in 1999 as a new member of the eukaryotic class II HDAC family (Verdel and Khochbin, 1999). Unlike most HDACs that are localized to the nuclei or both the cytoplasm and nuclei, HDAC6 localizes exclusively in the cytoplasm. As a 1215 amino acids protein with a molecular weight of 160 kDa, HDAC6 contains eight serine-glutamic acid repeats in the C-terminal domain and a nuclear export signal in the N-terminal domain, contributing to the cytoplasmic retention of HDAC6 (Bertos et al., 2004; Verdel et al., 2000). In addition, HDAC6 differs from other HDACs by harboring two catalytic subunits, both are located in the C-terminal domain and are essential for deacetylating substrates such as α-tubulin, cortactin, Hsp90, and peroxiredoxins (Parmigiani et al., 2008; Valenzuela-Fernandez et al., 2008; Zhang et al., 2006; Zou et al., 2006). With two motifs important for protein-protein interactions, i.e., the ubiquitin-binding zinc-finger (ZnF-UBP) and dynein-binding domain (Aldana-Masangkay and Sakamoto, 2011), HDAC6 has been reported to interact with a variety of proteins, from signaling molecules to cytoskeletal proteins and transcription factors (Valenzuela-Fernandez et al., 2008). The biological significance of these interactions is related to cell mobility, cell adhesion, protein degradation, immune response, or wound healing. One report indicates that HDAC6 is a component of stress granules, an aggregate of stalled protein translation apparatus, including messenger RNAs (mRNAs), eukaryotic initiation factors (eIFs), and 40S small ribosomal subunits (Kwon et al., 2007). Stress granules operate to preserve mRNA and translational machinery by serving as a temporary shelter. While addressing whether or not arsenite causes stress granule formation in human HaCaT keratinocytes, we found that HDAC6 relocalizes to the perinuclear space and regulated protein translation under arsenite stress.

**MATERIALS AND METHODS**

**Cell culture.** HaCaT keratinocytes or F65 normal human skin fibroblasts were cultured in high-glucose Dulbecco’s Modified Eagle Media (DMEM; Hyclone Laboratories) supplemented with 10% fetal bovine serum (FBS, SAFC Biosciences) and 0.1 mg/ml penicillin and streptomycin (Mediatech). Cells were grown to 80% confluency before treatment with sodium arsenite (Sigma-Aldrich), typically 30 min unless indicated.

**Immunocytochemistry.** Cells grown on glass coverslips were fixed in ice-cold methanol and washed in ice-cold phosphate buffered saline (PBS) followed by cell membrane permeabilization by 0.25% Triton X-100. Following 1 h blockage with 1% bovine serum albumin in PBS containing 0.1% Tween 20 (PBST), cells were incubated 1 h with primary antibodies against HDAC6, L36a, or S6 (Santa Cruz Biotechnology, 1:200 dilution). After five washes with PBST, the cells were incubated 1 h in dark with Alexa Fluor 488- or Alexa Fluor 568-conjugated secondary antibodies (Invitrogen Molecular Probes, 1:800 dilution). Following removal of unbound antibodies by five times washes in PBS, the coverslips were mounted onto a microscope slide with 10 μl of Prolong Gold antifade reagent containing DAPI (Invitrogen). Images were acquired under an Olympus fluorescence microscope with 100× lens.

**Isolation of ribosomes.** Ribosomes were prepared as described (Melamed et al., 2009). Briefly, HaCaT cells were collected with the ribosomal buffer (20mM Tris-HCl pH 7.4, 140mM KCl, 5mM MgCl₂, 0.5mM dithiothreitol, 0.1 mg/ml cycloheximide) and centrifuged for 15 min at 14,000 rpm (17,500 × g) to remove nuclei, mitochondria, and cell debris. The supernatant was loaded onto a 3 ml 10% plus 6 ml 35% sucrose gradient column for 4 h centrifugation at 4°C and 37,000 rpm (240,000 × g) to pellet ribosomes, which were resuspended in 100 μl Tris-EDTA buffer (200mM Tris, pH 8.0, 1mM EDTA) for measurements of protein concentration and Western blot analyses. For separation of ribosomal subunits, the supernatant after 14,000 rpm (17,500 × g) centrifugation was loaded onto a continuous 10, 20, 30, 40, and 50% sucrose gradient column for 3.5 h centrifugation at 4°C and 37,000 rpm (240,000 × g). The sucrose gradient columns were loaded to a BioLogic LP system (Bio-Rad) for collecting fractions every min at a flow rate of 0.5 ml/min while pumping 60% sucrose through the bottom of the column. Proteins in each fraction were precipitated using trichloroacetic acid and acetone and resuspended in 50 μl of 2× SDS-loading buffer for Western blot analyses.

**Western blot analyses.** Proteins were separated on a 10% SDS-polyacrylamide gel (SDS-PAGE) and transferred to an ImmunoBlot PVDF membrane (Bio-Rad). After blocking overnight at 4°C in Tris-buffered saline with Tween 20 (TBST) buffer (10mM Tris, pH 8.3, 150mM NaCl, 0.05% Tween 20) containing 5% milk proteins, the membrane was incubated 1 h with primary antibodies, 1:1000 dilution, against Nrf2, HDAC6, L36a, S6, α-tubulin (Santa Cruz Biotechnology), vinculin (Abcam), acetyl-α-tubulin, or acetyl-histone 2B (Cell Signaling). The membranes were washed five times with TBST and incubated with secondary antibodies conjugated with horseradish peroxidase (Invitrogen, 1:7000 dilution) for enhanced chemiluminescence (ECL) reaction and subsequent detection by ChemiDoc XRS+ imaging system (Bio-Rad) or film exposure (Kodak).

**Immunoprecipitation.** HaCaT cells were collected in lysis buffer (10mM Tris, 5mM EDTA, 50mM NaCl, 50mM NaF, 1% Triton X-100, and 1× protease inhibitor cocktail [BioVision]). HDAC6 antibody (400 ng, Santa Cruz Biotechnology) was added to cell lysates containing 50 μg of proteins for overnight incubation at 4°C with rotation. An aliquot of 25 μl of protein A/G bead in suspension (Santa Cruz Biotechnology) was added for additional 3-h incubation at 4°C with rotation. Proteins bound to antibodies-protein A/G beads were isolated by centrifugation at 4000 rpm (1431 × g) for 1 min. The beads were resuspended in 1 ml lysis buffer containing 1% Triton X-100 for four washes. Proteins were dissociated from the beads by boiling for 10 min in 25 μl of 2× SDS-sample loading buffer for Western blot.

**Two-dimensional SDS-PAGE.** Proteins were analyzed with the Zoom IPGRunner System according to manufacturer’s instruction (Invitrogen). Briefly, 100 μg of proteins were precipitated from cell lysates by methanol and chloroform and solubilized in 150 μl rehydration buffer (8M urea, 2% 3-[3-cholamidopropyl]dimethylammonio)-1-propane sulfonate, 0.5% vol/vol ZOOM Carrier Ampholytes, 20mM dithiothreitol, 0.002% bromophenol blue) by 1 h incubation at 37°C. Each protein sample (150 μl) was added to one lane in the ZOOM IPGRunner cassette. A ZOOM Strip pH 3-10NL was inserted into each well and incubated overnight to rehydrate the strips. The strips were focused according to the manufacturer’s recommendation, i.e., 200 V for 20...
min, 450 V for 15 min, 750 V for 15 min, and 2000 V for 30 min. The strips were equilibrated in buffer A (106mM Tris-HCl, 141mM Tris, 2% SDS, 10% glycerol, 0.51mM EDTA, 0.22mM bromophenol blue) for 15 min, transferred to buffer B (same as buffer A, except 50mM dithiothreitol was replaced with 125mM Iodoacetamide), and then washed once with ultra pure water. The strips were sealed onto a 10% SDS polyacrylamide gel with 0.5% agarose. The proteins were separated at 60 V for 150 min and visualized by SYPRO Ruby Staining (Invitrogen).

**RNA isolation and quantitative real-time reverse transcription-PCR.** RNA was extracted from whole cell lysates or ribosomal fractions with TRIzol (Invitrogen). Total RNA (1 μg) was reverse transcribed to complementary DNA (cDNA) with a RevertAid kit (Fermentas). Nrf2 mRNA was amplified using the primer pair of 5’-TGCTTTATAGCGTAAACCT-3’ (sense) and 5’-ATCCATGTCTTGACAGCAC-3’ (antisense) at 95°C for 15 min to activate the Taq polymerase and then 39 cycles of 95°C for 15 s, 60°C for 30 s, and 72°C for 30 s. After PCR, melting curves were acquired by stepwise increase of the temperature from 55°C to 95°C to ensure that a single product was produced by PCR. The level for 18s ribosomal RNA, serving as an internal loading control, was measured in parallel with the primers of 5’-TCCTTGGATGTTGGTAGCCGTTTCT-3’ (sense) and 5’-TCCTTGGATGTTGGTAGCCGTTTCT-3’ (antisense).

**Measurements of new protein synthesis.** Following 20 h serum starvation in DMEM with 0.5% FBS, cells were placed in methionine-free DMEM 1 h before arsenite treatment. L-Azidohomoalanine (AHA, Invitrogen) was added to the media during 30 min sodium arsenite exposure for incorporation of AHA into newly synthesized proteins. Cells were lysed in 50μM Tris-HCl pH 8.0 buffer with 1% SDS and were sonicated to solubilize proteins. After centrifugation at 12,000 rpm (13,000 × g) for 10 min to remove insoluble materials, 100 μg AHA-labeled proteins in the supernatant were used for the Click-it reaction, which occurs between an azide (AHA) and an alkyne-biotin (Invitrogen). Western blot analyses were performed to detect the newly synthesized proteins using streptavidin-conjugated horseradish peroxidase (Thermo Scientific) and ECL reaction.

**FIG. 1.** Arsenite induces perinuclear localization of HDAC6. HaCaT keratinocytes grown to 80% confluency on glass coverslips were exposed to 100μM sodium arsenite for 30 min. Immunocytochemistry was performed to determine subcellular localization of HDAC6 (green) and HuR (red). The images from the same cell were overlaid to determine colocalization of HDAC6 and HuR (yellow). Pictures were taken under 100× lens of an Olympus fluorescence microscope and enhanced 5X for showing stress granules (A and B). The number of HaCaT cells showing perinuclear localization of HDAC6 was counted from six random views at ×100 magnification (C). F65 normal skin fibroblasts grown to 80% confluency on glass coverslips were exposed to 10μM sodium arsenite for 30 min. Immunocytochemistry was performed to determine subcellular localization of HDAC6 (green) (D). The number of F65 cells showing perinuclear localization of HDAC6 was counted from six random views at ×100 magnification (E). *Indicates $p = 0.0001$ from untreated control by one-way ANOVA followed by Bonferroni post hoc test.
Statistics. Statistical analyses were performed using one-way ANOVA followed by Bonferroni post hoc test with GraphPad Prism software.

RESULTS

Sodium Arsenite Induces Subcellular Relocalization of HDAC6

HDAC6 was shown to associate with stress granules and played an important role in stress granule formation in HeLa cells and mouse embryonic fibroblasts (MEFs) (Kwon et al., 2007). When HDAC6 was tested for its subcellular localization during arsenite stress in human keratinocytes, we failed to detect HDAC6 in stress granules (Figs. 1A and B). HuR, a member of the embryonic lethal abnormal visual family of RNA-binding protein, has previously been shown to associate with stress granules during sodium arsenite stress (David et al., 2007). Immunocytochemistry staining for HuR showed stress granule formation by sodium arsenite treatment in HaCaT cells, serving as a positive control (Figs. 1A and B). The immunofluorescent signal of HDAC6 did not overlap with that of HuR, excluding stress granule localization of HDAC6 (Figs. 1A and B). Whereas HDAC6 was distributed evenly in the cytoplasm of untreated cells, treatment with arsenite caused perinuclear localization of HDAC6 (Fig. 1A). The percentage of cells with perinuclear localization of HDAC6 was scored following treatment with various doses of sodium arsenite from 1 to 200 μM. Sodium arsenite caused perinuclear localization of HDAC6 in 55–70% cells (Fig. 1C). We used F65 normal human skin fibroblast cells to demonstrate that HDAC6 relocalization is not limited to HaCaT cells. Arsenite at 10 μM caused perinuclear localization of HDAC6 in about 40% F65 fibroblasts (Figs. 1D and E), suggesting that the phenomenon of HDAC6 relocalization is not exclusive to the immortalized HaCaT keratinocytes. Therefore, sodium arsenite caused perinuclear distribution of HDAC6 instead of association of HDAC6 with stress granules.

HDAC6 Association With Ribosomes

The level of HDAC6 protein did not change in total cell lysates as a result of arsenite treatment (Fig. 2A). The perinuclear distribution of HDAC6 suggested its association with the endoplasmic reticulum (ER) where ribosomes reside. When ribosomes were prepared from cell lysates by ultracentrifugation, we found that treatment with sodium arsenite caused an increase in HDAC6 association with ribosomes (Fig. 2B). The ribosomes, each containing a 40S small subunit and a 60S large subunit, have a sedimentation efficiency of 80S and can form polysomes when bound to mRNA during the process of translation elongation. The 40S small subunit forms a preinitiation complex containing eIFs 1, 1A, 3, 5, and eIF2/GTP/τRNA{\text{Met}} ternary complex, resulting in sedimentation efficiency of 43S. To determine which form of ribosomes HDAC6 was associated with, we separated ribosomal subunits 40/43S, 60/80S, and polysomal fractions following ultracentrifugation through a sucrose gradient. In the ribosomal fractions from untreated cells, there was a baseline presence of HDAC6 at 40/43S fractions (Fig. 3). Arsenite treatment caused an increased level of HDAC6 in 40/43S and 60/80S fractions (Fig. 3). Although the presence of HDAC6 in 60/80S fractions was significantly elevated, HDAC6 was also present in the lighter polysomal fractions of arsenite-treated cells (Fig. 3). Small ribosomal subunit protein S6 and large ribosomal subunit protein L36a were used to correlate the fractions with ribosomal peak profiles as shown in the ultraviolet spectrum of liquid chromatography (Fig. 3). As expected L36a was absent in 40/43S fractions, while the presence of S6 indicated the fractions containing ribosomal small subunit, intact ribosomes, and polysomes.

To further demonstrate that arsenite caused an association of HDAC6 with ribosomes, we measured colocalization of HDAC6 with the ribosomal small subunit protein S6 or the large subunit protein L36a by immunocytochemistry. Cells stressed with sodium arsenite showed colocalization of HDAC6 with S6 or L36a (Figs. 4A and B). To demonstrate the physical interaction between HDAC6 and the ribosomal proteins, HDAC6 was immunoprecipitated from HaCaT cells.
for measurements of L36a or S6. With arsenite concentration at 1 μM or above, an increased association of HDAC6 with S6 or L36a was observed (Fig. 5). These results suggest that HDAC6 was recruited to and physically interacted with ribosomes due to sodium arsenite treatment in HaCaT cells.

Role of HDAC6 in Stress-Induced Protein Translation

The association of HDAC6 with the ribosomal complex led us to postulate a role of HDAC6 in protein translation during stress.

FIG. 3. Arsenite causes HDAC6 association with 40/43S and 60/80S ribosomal subunits. Cell lysates from HaCaT keratinocytes treated 30 min with 100 μM sodium arsenite were used to prepare ribosomal fractions via ultracentrifugation of 10–50% continuous sucrose gradient and liquid chromatography. Each fraction was used for precipitation of proteins and Western blot to measure HDAC6, S6, and L36a. The figure is from one experiment representative of three independent experiments.

FIG. 4. HDAC6 colocalizes with the ribosomal S6 and L36a proteins during arsenite stress. HaCaT keratinocytes were treated 30 min with 100 μM sodium arsenite for immunocytochemistry to detect the colocalization of HDAC6 (green) with ribosomal small subunit protein S6 (red, A), or with ribosomal large subunit protein L36a (red, B). Pictures were taken under the 100× lens of an Olympus fluorescence microscope.

FIG. 5. HDAC6 physically interacts with ribosomal S6 and L36a proteins during arsenite stress. HaCaT cells exposed to low and high doses of arsenite were used to immunoprecipitate HDAC6 for measurements of S6 and L36a by Western blot. The images are from one experiment representative of three independent experiments.
arsenite stress. Recent works from our laboratory have shown that oxidative stress induces de novo Nrf2 protein translation (Purdom-Dickinson et al., 2007a, b; Xu, Zhang, Sheveleva, Strom, Dinh, Lee, and Chen, unpublished data; Zhang et al., 2011). With arsenite treatment, Nrf2 mRNA levels did not change significantly; however, there was a clear induction of Nrf2 protein (Figs. 6A–C). When an mRNA strand is being translated, it is occupied by ribosomes. Isolation of ribosomes for measurements of Nrf2 mRNA showed an increased association of Nrf2 mRNA with ribosomes due to arsenite treatment (Fig. 6D). This indicated that Nrf2 mRNA was indeed recruited to the ribosomal complex during stress for Nrf2 protein translation.

To address the role of HDAC6 in de novo Nrf2 protein translation, we used Tubastatin A (TA), a cell permeable selective inhibitor of HDAC6 catalytic activity (Butler et al., 2010). Measurements of acetylated α-tubulin, the most studied substrate of HDAC6, confirmed the inhibitory effect of TA in HaCaT cells (Fig. 6B). There was no effect of TA on the acetylation state of histone 2B (Fig. 6B), indicating that TA was indeed selective for HDAC6 and did not inhibit other HDACs. Treatment of TA was able to prevent sodium arsenite from inducing association of Nrf2 mRNA with ribosomes and elevation of Nrf2 protein (Fig. 6B–D). Although TA caused a baseline elevation of Nrf2 transcript for an unknown reason, TA did not affect total Nrf2 mRNA significantly by arsenite treatment (Fig. 6A). These results suggest that HDAC6 participates in the regulation of stress-induced Nrf2 protein translation.

To address whether HDAC6 participates in regulation of additional proteins that undergo stress-induced de novo translation, we used a nonradioactive method to label newly synthesized proteins. As expected, arsenite or TA treatment caused an overall inhibition of protein synthesis (Fig. 7). Arsenite treatment increased the intensities of seven bands, with a molecular weight ranging from 15 to 55 kDa, whereas most of these inductions were blocked by TA treatment (Fig. 7). This data indicate a role of HDAC6 in arsenite stress–induced de novo synthesis of proteins in addition to Nrf2.
Two-dimensional gel electrophoresis was used to determine whether HDAC6 affected proteins that alter their levels in response to arsenite stress. Several protein groups showed increased intensities when HaCaT keratinocytes were stressed with arsenite (Figs. 8A and B). There were no changes in the group a or b between control and arsenite-treated cells (Figs. 8A and B). However, group c had four proteins with an increased intensity around 55–72 kDa at pH range 4–5 in the arsenite-treated cells (Figs. 8A and B). In addition, prominent changes can be seen in groups d (one spot at 20 kDa, pH 6.0), f (three spots at 70 kDa, pH 6.5), g (one spot at 37 kDa, pH 7.0), and i (one spot at 65 kDa, pH 8.5, Figs. 8A and B). When the protein patterns were compared with TA pretreated cells, TA alone had effects on basal levels of several groups of proteins (Fig. 8C). Nevertheless, HaCaT cells exposed to TA plus sodium arsenite had several protein groups that were differently expressed compared with the TA or arsenite treatment alone (Figs. 8B–D). TA plus arsenite treatment exhibited less changes in the group c, d, f, or i, compared with arsenite treatment alone (Figs. 8B and D). This suggests that although HDAC6 is involved in basal protein expression, it has a profound effect on selective protein translation during arsenite stress.

**DISCUSSION**

In this study, we found that arsenite stress caused HDAC6 to be associated with the ribosomes. Ribosomal fractionation, subcellular colocalization, and immunoprecipitation experiments demonstrated physical interactions of HDAC6 with the small and large ribosomal subunits. Catalytic inhibition of HDAC6 indicated a role of HDAC6 in arsenic stress–induced Nrf2 protein translation. Measurements of Nrf2 mRNA association with ribosomes suggest that HDAC6 regulated Nrf2 mRNA recruitment to ribosomes due to arsenite treatment. When new protein synthesis was measured, we found a role of HDAC6 in arsenic-induced de novo translation of proteins in addition to Nrf2. These results demonstrate a novel finding that arsenite stress causes perinuclear translocation of HDAC6, where HDAC6 interacts with ribosomes and regulates selective protein translation.

In our studies, perinuclear translocation of HDAC6 was found with a wide range of arsenite concentration, from 1 to 200μM. Most arsenic studies have focused on low doses of sodium arsenite (< 10μM) for extended periods. Such experimental protocol is relevant to human exposure, because Environmental Protection Agency guideline states the maximum containment level is 10 μg/l (133nM). However, many countries have reported concentrations of arsenic in drinking water ranging from 150 μg/l (2μM) to greater than 600 μg/l (8μM) (Lubin et al., 2007). In addition to the environmental source of arsenic, arsenic trioxide has been used as a chemotherapeutic agent for treatment of leukemia at a dose range of 0.10–0.15 mg/kg (101–150μM) daily for 25–60 days (Soignet et al., 1998). Whereas chronic exposure to low-dose arsenic may contribute to skin diseases and cancer, acute exposure to high doses can cause diseases involving liver, kidneys, and...
HDAC6 in Stress-Induced Protein Translation

cardiovascular systems. Therefore, understanding the effects of low or high doses of arsenic exposure is important for the etiology of diseases consequential to arsenic exposure. Our data demonstrate that HDAC6 is recruited to the ribosomal complex at both low and high concentrations of arsenite.

HDAC6 has been reported as a component of stress granules. The observation of HDAC6 being associated with stress granules was documented with HeLa and MEF cells following exposure to 1mM sodium arsenite for 60 min (Kwon et al., 2007). Both the deacetylation activity and ubiquitin-binding domain of HDAC6 participate in assembly of stress granules (Kwon et al., 2007). In our study with HaCaT keratinocytes, although we found stress granules due to 30 min exposure to 100µM sodium arsenite, HDAC6 was not detected in the stress granules visible under a microscope (Figs. 1A and B). Two enlarged views show nonoverlapping signals of HDAC6 with HuR, a stress granule marker (Fig. 1B). Stress granules undergo dynamic size changes as multiples of 48S. Because HDAC6 was detected in 40/43S fractions of ribosomes (Fig. 3), and stress granules are aggregates of translation initiation complex containing 40S small ribosomal subunits, it is possible that HDAC6 may be present in small stress granules that were beyond the detection limit of a regular fluorescence microscope. If HDAC6 is present in the stress granules visible under a microscope after immunochemical staining, it may appear as large ribosomal aggregates showing similar sizes as heavy polysomes. The fact that HDAC6 was more abundant in 40/43S to 60/80S fractions than polysomal fractions is consistent with our immunocytochemical data showing the absence of HDAC6 in the stress granules.

HDAC6 is best known for its association with the cytoskeleton and regulation of cytoskeletal organization through deacetylation of α-tubulin (Hubbert et al., 2002). It is possible that deacetylation of α-tubulin resulting in remodeling of the microtubule network, allowing HDAC6 to be shuttled to the ER where it becomes associated with ribosomes. Several reports have shown that ribosomes can bind to microtubules and actin filaments (Hamill et al., 1994; Hesketh, 1994; Zhou and Rabinovitch, 1998). In addition, critical components of the translational machinery, i.e., aminoacyl-tRNA synthetase, eukaryotic initiation factors (eIF2, eIF3, eIF4, and eIF6), and eukaryotic elongation factors (eEF1α, eEF1β, eEF1γ, and eEF2) have physical interactions with the cytoskeleton (Kim and Coulombe, 2010). Such interaction is important for protein translation (Ainger et al., 1993; Taneja et al., 1992). Therefore, HDAC6 may serve as an auxiliary protein, bridging the interaction between the cytoskeleton and translational machinery.

Several reports indicate that HDAC6 can be phosphorylated and deacetylase activity can be modulated depending on which amino acid residue is phosphorylated. Deacetylase activity of HDAC6 is inhibited when the epidermal growth factor receptor phosphorylates tyrosine 570 or glycogen synthase kinase 3β (GSK3β) phosphorylates serine 22 (Deribe et al., 2009). In contrast, protein kinase CK2 phosphorylates serine 458 on HDAC6, resulting in an increased deacetylases activity (Watabe and Nakaki, 2011). Arsenite stress has been shown to cause activation of PI3K (Ding et al., 2009; Shack et al., 2003), which in turn activates Akt and causes inhibition of GSK3β consequentially. Because GSK3β inhibits HDAC6, inhibition of GSK3β due to arsenite treatment could contribute to activation of HDAC6. The role of posttranslational modifications and the deacetylase activity of HDAC6 in its interaction with the ribosomes remain to be determined.

Our study suggests that HDAC6 regulates arsenite stress–induced protein translation. The intense signals of HDAC6 from 40/43S to 60/80S fractions suggest a role of HDAC6 in translation initiation instead of translation elongation. Translation initiation is the rate-limiting step for the process of protein translation. For 95–97% mRNA species, translation initiation is mediated by eIF4E recognition of 7-methyl guanine cap at 5’ end of the mRNA species. Such 5’ 7-methyl guanine cap-dependent translation initiation is inhibited during stress. As a result, numerous genes are inhibited for translation into proteins. With arsenite stress, a well-characterized example is inhibition of cytochrome p450 3A23 protein synthesis (Noreault et al., 2005).

Selective protein translation occurs under stress conditions, often through the Internal Ribosomal Entry Site (IRES) in the 5’ untranslated region of the mRNA species. The interaction of IRES transactivating factors results in recruitment of eIFs and ribosomes for translation initiation. Recent work from our laboratory has identified several RNA-binding proteins responsible for recognition of Nrf2 mRNA to be translated into Nrf2 protein under oxidative stress (Zhang et al., 2011). In this scenario, HDAC6 may regulate an intermediate protein that “shuttles” Nrf2 mRNA to ribosomes for translation initiation. In addition to regulating mRNA association with translation apparatus, HDAC6 may deacetylate ribosomal proteins. Many ribosomal proteins are acetylated during normal biochemical metabolism (Zhao et al., 2010). The recruitment of HDAC6 to the ribosomal complex may be critical for deacetylating the ribosomal proteins essential for translation initiation. Although the mechanism of stress-induced protein translation remains largely unknown, our finding with HDAC6 adds a new piece of information for solving the puzzle of stress-induced protein translation.

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